Animal models of IBD

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Murine models of IBD 2006

1. Spontaneous Models
   Cotton top tamarin, C3HBir Maus, SAMP/Yit

2. Inducible models/ Hapten reagents
   DSS, PG-PS, Oxazolone, acetic acid
   TNBS, DNBS

3. Adoptive transfer models
   CD45RBhigh model, CD62L+ model, Tgpsilon tg mouse

4. Genetically engineered models
   Transgenic mice: TGFbeta-dom.neg.RII, STAT4, IL-7
   Knockout mice: IL-2, IL-10, TCR, MDR1, NF-kappa
   Bp50, DARE Maus
   Conditional Knockout mice: STAT3 in macrophages
# Mouse models of colitis

<table>
<thead>
<tr>
<th>Model</th>
<th>Pathology</th>
<th>Site</th>
<th>Pathogenesis/Remarks</th>
<th>Costs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spontaneous model</td>
<td></td>
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<tr>
<td>SAMP/Yit</td>
<td>chronic</td>
<td>ileum</td>
<td>activation of Th1 T cells towards luminal antigens in the ileum only</td>
<td>high</td>
</tr>
<tr>
<td>Inducible models</td>
<td></td>
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<tr>
<td>DSS</td>
<td>acute, chronic</td>
<td>colon</td>
<td>toxic intestinal damage plus activation of the mucosal immune system (TH1/TH2)/activation of the immune system (TH1-mediated; hapten-specific)/optimization of TNBS dosage required, only certain strains</td>
<td>low</td>
</tr>
<tr>
<td>TNBS</td>
<td>acute, chronic</td>
<td>colon</td>
<td></td>
<td>low</td>
</tr>
<tr>
<td>Adoptive transfer model</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>CD45RB CD62L+ cell transfer into SCID mice</td>
<td>acute, chronic transmural</td>
<td>colon, duodenum</td>
<td>IL-12 driven TH1 T cells/induction of colitis requires 6-12 weeks, appropr. animal facility for SCID mice required</td>
<td>high</td>
</tr>
<tr>
<td>Genetically eng. model</td>
<td></td>
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<tr>
<td>IL-2 knockout mice</td>
<td>acute, chronic</td>
<td>colon</td>
<td>IFN-gamma producing T cells/colitis 6-15 weeks after birth, variability between mice TH1 T cells in response to bacterial antigens/requires immunization of mice</td>
<td>medium</td>
</tr>
<tr>
<td>STAT-4 transgenic</td>
<td>acute, chronic</td>
<td>colon, ileum</td>
<td></td>
<td>medium</td>
</tr>
</tbody>
</table>
Readout parameters in murine models of IBD

- weight curves
- Histology
- Cell isolation: cytokine, FACS, proliferation/apoptosis
- endoscopy

Grossly normal mucosa
Methylene blue stained grossly normal mucosa

Pathogenesis of mouse models

1. Bacterial flora
   Germfree mice fail to develop colitis (Sadlack 1994)
   Monoassociation with certain strains induces colitis (Rath 1998)
   Antibiotics suppress colitis activity (Herfarth 1998)
   T cells in the lamina propria react to their autologous commensal flora (Duchmann 1996)
   Adoptive transfer of flora specific T cells induces colitis (Cong 1998)

2. Key cellular players
   Macrophages (STAT3 conditional KO)
   T lymphocytes (adoptive transfer, T cell depletion)
   - CD4+
   - CD8+
   - Treg
   B lymphocytes (NFκB p50 KO)
   Epithelial cells
A key role for IEC in the onset of ileitis in SAMP/Yit mice

Olson et al. JEM 2006
Changes in claudin2 and occludin in SAMP/Yit mice

Occludin ileum

Occludin colon

Olson et al. JEM 2006
Epimorphin controls crypt proliferation

- Epimorphin regulates epithelial mesenchymal interactions
- Epimorphin deficient mice were generated
- This protein regulates crypt cell proliferation both in the small and large intestine
- Proliferation is controlled via activation of BMP/TGF-beta

Crypt proliferation protects from experimental colitis

WT KO

WT KO

Dendritic cells: Key players in gut inflammation

Communication between bacteria and the mucosal immune system via DCs

Hypo responsiveness, Mucosal Compartmentation, Tolerance

CD11c

Dendritic cells

Intestinal and MLN dendritic cells regulate colitis activity

CD8α+, CD11b+ CD11c+ CD103+

Annacker et al. JEM 2006
Basic concepts in IBD animal models

Pathogenic

Antigens

Dendritic cells

TNF, IFN-γ

TH1

CD4

TH2

IL-5, IL-13

Protective

FoxP3

Treg

contact

IL-10

Tr1

TH3

TGF-β

CD4

circulation

TH1 and Th2 cytokines

Tr1 and Th3 cytokines

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Immunopathogenesis of colitis: Decision making by APCs/dendritic cells

Bacterial Antigens

Dendritic Cells

High IL-12
Low EBI3

T helper 1

Low IL-12
High EBI3

IL-4/5, IL-13

Nieuwenhuis et al. PNAS 2003
Anti-IL-12 p40 antibodies induce T cell apoptosis

Anti p40 antibodies suppress established Th1 colitis via induction of T cell apoptosis (JEM 1995, Gastro 1999)

P19 transgenic mice exhibit multiorgan inflammation including GI tract (JII 2001 166 7563)

P19 but not p35 knockout mice fail to develop EAE (Nature 2003 421 744)
IL-23 is expressed in lamina propria DCs of mice with transfer colitis.
Generation of IL-23 p19 knockin mice

IL-23p19 wildtype

IL-23p19 targeted

wtexon1

+/-

wildtype

+/-

targeted

-/-
IL-23 KO mice are highly susceptible to inducible colitis models.
IL-23 KO mice are highly susceptible to TNBS induced colitis
IL-23 KO mice are highly susceptible to TNBS induced colitis

Wild-type

IL-23 KO

histologic score

wt KO

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Crossregulation of IL-12 p35 and p40 expression by IL-23 p19
Anti-IL-12 therapy rescues p19 knockin mice from lethal colitis

**Graph:**
- **x-axis:** Days
- **y-axis:** weight in %
- **Legend:**
  - Control
  - Anti-IL-12 p40

**Figure:**
- **Image 1:** PBS
- **Image 2:** Anti-IL-12 p40

**Text:**

Anti-IL-12 therapy rescues p19 knockin mice from lethal colitis.
A role for both IL-12/IL-23 in Crohn’s disease

- p35/p40 (IL-12)
- p19/p40 (IL-23)
- Bacterial antigens
- LPDC
- IEC
- T helper 1
- Memory responses Perpetuation
- T helper IL-17
- T cell survival
- Crohn’s disease

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IL-23 p19 controls colitis activity in IL-10 KO mice

IL-23 induces a T cell subset producing IL-6 and IL-17
Blocking IL-6 and IL-17 in experimental colitis

A role for IL-12/IL-23 in colitis?

naive → TGF-β

- → IL-12
+ → IL-4

IL-4 stabilizes TH2

naive → TGF-β

+ → IL-6, IL-1β

IL-6 stabilizes TH17

TH1 activation

TH2

iTreg

TH_{IL17}

IL-23 stabilization

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Anti-IL-12 p40 antibody therapy in Crohn’s disease

- 79 patients with active Crohn’s disease (CDAI 220-450)
- Randomisation: Placebo or 7 weeks 1 or 3mg/kg ABT874 s.c.: Induction of T cell apoptosis
Induction of T cell apoptosis via mTNF

A  

<table>
<thead>
<tr>
<th></th>
<th>Infliximab</th>
<th>Etanercept</th>
<th>c17-1a</th>
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<tbody>
<tr>
<td>active caspase 3</td>
<td>- - - + + + - - -</td>
<td>- - - - + + + -</td>
<td>- - - - - - + +</td>
</tr>
<tr>
<td>β actin</td>
<td>17 kDa</td>
<td>43 kDa</td>
<td></td>
</tr>
</tbody>
</table>

B  

- Graph showing Caspase 3 activation (relative to 0 hrs) at 24 and 48 hrs with different treatments.

References:
- Van den Brande et al. Gastroenterology 2003
- Matsuoka et al. Gastroenterology 2005
Blockade of the IL-6/ sIL6R system induces apoptosis in colitis
Blocking the IL-6 signaling pathway in Crohn’s disease

before MRA

after MRA

Ito et al. Gastroenterol. 2004
Clinical therapy of IBD and T cell apoptosis

Anti-TNF/IL-6R antibodies

Induction of T cell apoptosis (cell death)

- rapid

- delayed

T cell apoptosis

Azathioprine/ Rac1

6-MP responsiveness correlates with the induction of apoptosis

Tiede et al. J. Clinical Invest. 2003
A molecular mechanism of action of azathioprine

**CD28**

Rac1/2

vav

Rac-thioGTP

ERMT cell-APC conjugation

Inhibition of T cell-APC conjugates

**STAT3**

**IκB**

**NF-κB**

**Bcl-xL**

**Induction of apoptosis**

**azathioprine**
Azathioprine blocks vav activity on Rac1

6-Thio-GTP \(\rightarrow\) Vav \(\rightarrow\) Rac1-Thio-GDP \(\uparrow\uparrow\) \(\rightarrow\) Rac1-Thio-GTP \(\downarrow\downarrow\)

Apoptosis, Inhibition of T cell-APC conjugation
Steric modelling of ThioGTP

Cyan = switch 1 region of Rac1 (residues 27-35)
Magenta = switch 2 region of Rac1 (residues 59-71)
Yellow = contact area of vav1 with Rac1
Ulcerative colitis and colon cancer

Risk factors for cancer: duration and extent of disease, flares, PSC
Chronic intestinal inflammation favours cancer development

IL-10 knockout Colitis

Early TH1 (IFN-gamma) Colitis
Late TH2 Colitis with colon cancer
Tumorigenesis in the AOM/DSS model
IL-6 blockade suppresses tumorigenesis in the DSS/AOM model

Control antibody

Anti-IL-6R Ab

Becker et al. Immunity 2004
Shift from mIL-6R expression towards sIL-6R during tumorigenesis
Blockade of the sIL-6R suppresses tumorigenesis
How does colitis drive tumor growth?
Summary

• There are numerous experimental IBD models
• There is no ideal model: the choice of the model depends on the questions of the investigators
• Pathogenesis: The intestinal flora drives gut inflammation in most IBD models
• Many models have early defects in IEC
• Additional key players are DCs and T cells
• The data have important implications for designing novel therapeutic approaches for IBD: in particular, targeting of cytokine signal transduction and T cell apoptosis emerge as important approaches for therapy of IBD
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